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ON THE

MEDICAL RESEARCH INSTITUTE

FOR THE YEAR

1913.

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TROPICAL MEDICINE

Annual Report on the Medical Research Institute for the year 1913.

INTRODUCTORY.

1. A large amount of original research has been done during the year 1913, and, as in previous years, the Medical Officers have sent a generous supply of material from their stations. Specimens have been received from the Gambia, the Gold Coast, Sierra Leone and from Northern Nigeria, as well as from Southern Nigeria.

2. Much of the material thus received, has been made use of in research work, which is the primary object of the Institute, and the Museum has been enriched by the addition of numerous collections of biting insects, snakes, helminths, and pathological exhibits generally, including photographs.

OFFICERS IN CHARGE.

3. Dr. A. Hutton was Acting Director from the beginning of the year until the end of April, when he left to take up duties under the Yellow Fever Commission, at Accra. Dr. J. W. Scott Macfie was in charge from the beginning of May until 24th October, and Dr. J. E. L. Johnston acted as Assistant Bacteriologist from 18th June until the middle of October. I assumed duty as Director on 24th October. A few days after my arrival, Dr. Macfie proceeded to Eket, to continue his work on the Nigerian trypanosome, which he had previously described.

THE WORK DONE.

4. This is divided into A, Original Research, and B, Routine Examinations.

A.—ORIGINAL RESEARCH.

HELMINTHIASIS.

5. Dr. A. Hutton has kindly furnished the report which is reproduced below. It deals with the work which he did with Dr. Leiper on Helminthiasis generally, and on the insect host of Loa loa, in particular:—

> 6. "Early in January, Dr. R. T. Leiper, Helminthologist to the London School of Tropical Medicine, arrived at Yaba from Calabar where he had been carrying out researches regarding the transmitting agent of Loa loa.
>
> 7. "Dr. Graham, the late Director, had reported that certain wells in Lagos and Ebute Metta were infected with Ankylostomes, and Dr. Leiper had been requested to enquire into their alleged presence. After a careful examination of the wells, and also of certain preparations in the Institute Museum, he came to the conclusion that the so-called Ankylostomes were really free-living nematodes, which, instead of being harmful, might be said to 'exercise an insignificantly useful function as scavengers of the wells.'

8. "The blood of the patients in the adjoining Lunatic Asylum was examined for the presence of larvae of Loa loa, with a view to continuing the work on the development of that parasite, but although a considerable number were found to be infected, lack of biting flies, different from those with which he had already worked, prevented his taking advantage of them. An opportunity occurred of making a *post-mortem* examination on one patient, and calcified Filariae were found in the testicle and lung.

9. "An attempt was made to infect Stomoxys calcitrans and S. nigra, which were easily obtained from the neighbouring villages, and Hippobosca, caught on cattle, with the filaria of a dog, but the result was negative.

10. "In February, Dr. Leiper went to Kano, in Northern Nigeria, and the Acting Director accompanied him. The object of this visit was to examine the ostriches, which are kept there, by the Education Department. It is proposed to have ostrich-farms on a large scale in the Colony, and it is thus of great importance that the birds should be free from any disease, which would tend to increase, when they are herded together in large numbers. The ostriches then in captivity were found to be free from intestinal parasites. South African farms suffer heavy losses annually owing to this cause, but seem to ignore it, for they not long since imported a considerable number of birds from Northern Nigeria, with the object of improving the breed by the introduction of fresh blood. These birds might be free, when imported, from parasites, but they are certain to become infected when mixed with the other birds on the farms.

11. "Other animals were similarly examined. Practically all the horses were found to harbour parasites of some kind, but not in such numbers as to affect their health. No opportunity occurred of making a *post-mortem* examination on any horse, and it was thus impossible to ascertain the actual parasites present.

"Cattle, sheep, and goats were examined daily at the slaughter place. The goats were singularly free from infection, but the cattle and sheep had considerable numbers. Apparently this does little harm, for the animals were in excellent condition and the meat was of excellent quality.

12. "House-flies are a veritable plague in Kano, due probably to the universal presence of horses in the compounds and to the practical impossibility of dealing effectively with their droppings. Biting flies were represented only by Hippobosca maculata, but these were in great numbers.

13. "Lokoja was the next place visited, and there many cases of Onchocerca volvulus were found amongst the prisoners. Much interesting and important work was done to find the fly which transmits this disease, but as Dr. Leiper has reported elsewhere on the experiments, no more need be said about them here.

"It may be remarked, however, that the complete absence of some flies and the great scarcity of others was a continual handicap throughout the trip and nowhere was it more marked than in Lokoja.

14. "On the way to Sapele, a stop of a few days was compulsory at Forcados. Here several specimens of Chrysops longicornis were captured, and an attempt was made, with negative results, to infect them with the larvae of Loa loa.

"These negative results are interesting when it is remembered that C. silacea and C. dimidiata are the carriers of that parasite. Several other experiments were done with species of Tabanus and these were also negative.

15. "On arrival at Sapele, we were much disappointed to find that no Chrysops were available. A fruitless search was made at Messrs. Miller Bros. Rubber plantation and also at Ologbo. At certain seasons of the year, these insects, locally known as 'Red flies,' are very common and are a greater pest than mosquitoes. This is especially the case at the rubber plantation, and here an attempt was made to find their breeding places, but with no success. A number of Tabanus sp. and one Hæmatopota sp. were fed on an infected case, but no development took place.

16. "During the journey back to Lagos through the creeks, the experiments were continued with the flies captured *en route*.

"Numerous tsetse flies were captured throughout the journey. In some places they were very persistent, and several were caught biting after dark. Mosquitoes, especially Mansonioides, abounded at every place where we stopped for the night."

17. Dr. Johnston, in a paper, "Observations on Variations in Form, of Microfilariae found in Man," in the Annals of Tropical Medicine and Parasitology, Vol. VIII, No. 1, 21st April, 1914, pp. 73 to 79, mentions first the sheathless embryos which have so far been found in man. He then gives the symptoms, etc., of the patient in whose blood he found the embryos which he describes. The patient was a male Ndoma pagan, in the Bassa Province of Northern Nigeria. The symptoms were a lumbago-like pain, and indefinite pains from the knees downwards. The signs were a firm oedema of both feet and legs, and a differential leucocyte count of Polymorphonuclears, 60%, Eosinophils, 3%, Mononuclears, 3%, Lymphocytes 34%, and the Haemoglobin was 60%.

18. Filarial embryos of three types were found in the living blood. (A), Sheathless, actively motile, tapering posterior extremity, blunt tail, length 83 to 170 microns. (B), Resembling (A) closely, but having a sharply-pointed tail, total length 230 to 251 microns. (C), Sheathed embryos, apparently those of Loa loa.

19. Types (A) and (B) are then described. The "central viscus" of Manson was not observed. Both types presented very definite nuclear breaks. Tables of measurement of these are given.

Type (A) possessed coarse nuclei and there were usually three abreast in the greater portion of the body. The anterior extremity was devoid of nuclei for a distance of 2 to 6 microns.

The first break was narrow, but stretched right across the body.

The second break was also complete. The third was small, wider than the first but incomplete, and the fourth varied.

Type (B) possessed coarser nuclei than (A), averaging two abreast. The clear anterior extremity was 5 to 7 microns in length. The first break was wider than in type (A) but was seldom complete. The remaining breaks were relatively small and mostly incomplete.

20. Tables and a chart are given, and the measurements therein recorded do not agree with those of Acanthocheilonema perstans, but the author suggests that the embryos he describes may be merely variations of Acanth. perstans. He was unable to obtain adult specimens.

21. Dr. Jonhston has also published a paper, "A Note on Helminthiasis in Bassa Province, Northern Nigeria," in the Lancet, 27th September, 1913.

22. The physical features of the Province, and the climatic conditions are given shortly and a note concerning the people is added. The stools were examined in thirty cases, taken at random. There was a single infection with helminth ova in twelve, a double infection in six, triple in three, and no infection in eleven. The ova observed were Ankylostome in fourteen, Ascaris in six, Taenia solium in four, and Trichuris in three.

23. A table is given, showing the ova found and the physical condition of each patient. The blood of twenty-six was examined. Eosinophilia was a constant feature. A table is given showing the differential leucocyte count and the percentage of Hæmoglobin in each case. There is a short note as to treatment.

The view is expressed that the Bassa pagans are more frequently infected with Ankylostomes than are the Hausas, and this is attributed to the use by the latter of deep cement-lined pits for defaecation, whereas the pagan tribes have no latrine system.

24. The absence of symptoms is noted in many of the cases that showed an infection with Ankylostomes.

(*b*) TRYPANOSOMIASIS.

25. Perhaps the most important work was by Dr. Scott Macfie. "On the Morphology of the Trypanosome (T. nigeriense, n. sp.) from a case of Sleeping Sickness from Eket, Southern Nigeria."

This was published in the Annals of Tropical Medicine and Parasitology, Vol. VII, No. 3A, 11th August, 1913, pp. 339 to 356. The history of the discovery of Sleeping Sickness in the Eket district is given. Various observations on the disease in Northern Nigeria by the author are noted, and the reasons which led him to specially investigate the Eket cases are described. A full description of Sleeping Sickness in the Eket district follows, embodying many interesting facts.

26. The late Dr. Foran sent, to the Medical Research Institute, a guinea-pig which he had inoculated with the cerebro-spinal fluid, on March 5th, 1913, from a case under his charge in the Sleeping Sickness Camp at Ikotobo. Dr. Macfie's paper deals with the morphology of the parasite which developed in the guinea-pig.

27. This morphology is given in some detail under two headings.

A.—Living, unstained.—The elongated tapering body is of almost homogeneous consistency. The micronucleus can sometimes be distinguished as a small refractile body. The movements are active, but some individuals which are stouter than the average are relatively sluggish. The long slender forms vibrate extremely actively and move rapidly. The translatory movement appears to be spasmodic.

As a rule the flagellar end moves forward in the translatory movements. This end is attenuated, and the non-flagellar end blunt. The undulating membrane is conspicuous. Changes occur in blood which has been shed for some time. Then, the long slender forms of the parasite show a lessened activity, the non-flagellar end appears to become rounded off, and eventually the body becomes spherical.

28. B.—*Fixed and stained.*—The blood films were fixed for a definite time in Absolute Alcohol or in Osmic Acid and stained with Giemsa's solution. The longest trypanosome was 32 microns, and the shortest 8 microns. The breadth at the widest part varied between 1 and 2·5 microns. The parasites were polymorphic. Long slender, short stumpy, and intermediate forms occurred, and their relative proportions varied considerably from day to day. As a rule the long slender forms predominated, and these were of two types. The blepharoplast was terminal in one and the nucleus elongated, whilst in the other the blepharoplast was 1 micron from the blunt-pointed, snout-like posterior extremity. The intermediate forms varied in size and aspect, some being pointed at both ends, and others having a blunt, rounded posterior extremity with the blepharoplast either terminal or subterminal. The free flagellum was very short. The stumpy forms, exceedingly short in some instances, were relatively broad. They were characterised by a short free flagellum, a narrow membrane, a blunt posterior end, a large nucleus sometimes placed at the extreme anterior (flagellar) end, and a blepharoplast situated slightly subterminally. Trypanosomes with the flagellum free in its entire length were observed from time to time. These were in the long slender forms, with the blepharoplast some distance from the extremity and anteriorly drawn out into a filamentous termination. Ordinarily, the protoplasm appeared homogeneous. Both granules and vacuoles were observed in films fixed in osmic acid and overstained. The granules were coarse, and on both sides of the nucleus. The nucleus mostly was oval. It was rounded in the stumpy forms. It was near the centre of the body in most of the parasites, but sometimes, and in these instances always in the stumpy forms, it was anteriorly placed.

The blepharoplast was always round and deeply staining. The undulating membrane, was ample and thrown into folds in the long slender forms. Sometimes it was apparently absent. It was less folded in the stumpy and intermediate forms. The flagellum stained deeply and was always well-marked.

29. Tables of measurement and a chart are given, and these are compared with the *data* obtained by Stephens and Fantham for T. gambiense and T. rhodesiense.

30. The results of sub-inoculations into other animals by the author and by Dr. Foran are given.

31. The summary and conclusions of the paper contain the statements that the trypanosome under discussion seems to differ in several respects from T. gambiense. The form of Sleeping Sickness produced in man is mild, and is most common in young people. The parasites are few in the peripheral blood. The strain is but slightly pathogenic to the smaller laboratory animals. The trypanosome is smaller than T. gambiense. There is a constant appearance of very small forms in the circulation. The cell-protoplasm when well-stained is homogeneous. Some of the short stumpy forms have the nucleus placed far at the anterior (flagellar) end. It is also remarkable that a few parasites occur in which the flagellum is free in its entire length.

32. It is concluded that the trypanosome cannot be regarded as belonging to the same species as T. gambiense, and the name T. nigeriense is proposed for it.

33. Dr. Macfie in a later paper "On the Pathogenicity of the Trypanosome (T. nigeriense) from a case of Sleeping Sickness from Eket, Southern Nigeria," published in the Annals of Tropical Medicine and Parasitology, Vol. VIII, No. 1, 21st April, 1914, pp 29 to 39, again calls attention to the low degree of virulence of T. nigeriense when inoculated into the smaller laboratory animals. All the first experiments were made with the trypanosome obtained from the guinea-pig sent by Dr. Foran and already alluded to in the paper epitomised above. After the death of this guinea-pig on the 158th day, blood from other two infected guinea-pigs and an infected monkey was used. All the inoculations, two excepted, were subcutaneous and small amounts of blood, diluted with 1% Sodium Citrate solution, were used. Trypanosomes were scanty in most of the infected animals, and parasites of the small size characteristic of the strain, were seen in all the cases in which Trypanosomes developed in moderate numbers. No symptoms of illness were observed in any instance, and in every case the infection followed a chronic course.

34. Eight experiments were performed on rats, two black and six white. Seven were negative. A single trypanosome was found in one animal on the 59th day and the rat was alive and well after 69 days.

35. Inoculations were made into seven of the local mice. Three of these experiments were successful. Parasites were first seen on the 5th, 7th, and 9th day respectively. Two of the mice were accidentally killed (this is a frequent result of handling these very small animals), one on the 11th and the other on the 59th day. The third was alive after 107 days.

36. Twelve guinea-pigs were experimented with. Three became infected, one on the ? day, and the other two on the 27th day. The parasites were generally scanty.

37. Seven native dogs were inoculated, and only one became infected. Parasites first appeared in the blood of this animal on the 85th day, and the animal was well on the 115th day.

38. Two experiments were performed on monkeys. They were of different species, and both became infected, one on the 8th, and the other on the 11th day. One was alive on the 118th, and the other on the 137th day.

39. Two native goats were inoculated. One became infected, trypanosomes appearing in the blood on the 63rd and the 65th day. This goat was still alive and well on the 149th day.

40. These experiments indicate the slight degree of pathogenicity of the Nigeria trypanosome. Monkeys, guinea-pigs and mice appear to be more susceptible than dogs, rats and goats, and the last two seem to be the most resistant.

41. The results of sub-inoculations of T. gambiense, by (1) Yorke, (2) The Royal Society Commissioners in Uganda, (3) Thomas and Linton, (4) Laveran, (5) Brumpt and Wurtz, (6) Beck, (7) Bentmann and Gunther, are given and compared with the author's results. The comparison bears out the contention, that the strain of T. nigeriense used by Macfie is much less pathogenic to practically all the animals experimented with than T. gambiense.

42. Dr. Macfie has also a paper, "Preliminary Note on the Development of a Human Trypanosome in the Gut of Stomoxys nigra," in the Annals of Tropical Medicine and Parasitology, Vol. VII, No. 3b, 7th November, 1913, pp. 359 to 361.

43. The experiments of a number of other observers who worked with Stomoxys as a transmission-agent in Trypanosomiasis are quoted. Macfie succeeded in breeding S. nigra in captivity and hoped to investigate its rôle, but his experiments were interrupted.

44. He fed a number of Stomoxys caught in the laboratory, on a guinea-pig which was infected with the trypanosome which he describes as T. nigeriense. Thirteen flies were dissected from one to six days after their infecting feed. Twelve flies that had not fed on the infected animal were dissected as a control. No flagellates were found in any of the latter, but six of the former showed Herpetomonas in the gut.

45. Two further experiments were then performed. A fly that had hatched on the previous day was fed on the infected guinea-pig. Three days later the fly was dissected, and Herpetomonas were found in the mid-gut. In the second experiment performed in the same way, the fly died in four days after the infecting feed, and Herpetomonas were found in the mid-gut. No flagellates were found in the salivary glands or in the proboscis of either of the insects. Two other S. nigra that had been bred out but had not fed on the infected animal were dissected, and no flagellates were found.

46. Some interesting experiments were performed by Dr. Macfie and Dr. Johnston on various trypanosomes. Their results are published as "Observations on the Action on Trypanosomes of certain Drugs and of Staphylococcus pyogenes," in the Journal of the London School of Tropical Medicine, Vol. 11, Part III, pp. 207 to 212. A few initial experiments were made with dilute solutions of Iodine, Bromine, and Osmic Acid on trypanosomes in cover-slip preparations. Thereafter, separate and accurately-conducted experiments were carried out.

47. The trypanosomes employed were T. vivax in a sheep, and T. lewisi in a rat. The indicator used, styled the "trypanosome index," was obtained by counting the number of the parasites relative to 100 leucocytes (at least 200 leucocytes were counted in every case).

48. The Iodine solution used was, Tinct Iodi. minims VII. Aq. destillata ad one drachm.

49. *Experiment I.—Rat 41.*—Minims XX of the solution used on first day. Daily doses thereafter were minims X. Index remained high, but irregular.

Experiment II.—Sheep 65.— One drachm pure Tinct. Iodi. introduced intravenously. Death occurred on the fifth day.

The Bromine solution used was—Brom. (25%) minims VII. Aq. destillata ad one drachm.

50. *Experiment III.—Rat 42.*—The index remained stationary during the seven days of injection, with a twenty-minim dose on the first day, and ten-minim doses afterwards.

The Osmic Acid solution used was—Ac. osmic. minims XII. Aq. destillata ad one drachm.

51. *Experiment IV.—Rat 43.*—Ten-minim doses. Died in four days. Intense leucocytosis, great destruction of leucocytes, phagocytosis of red cells and of trypanosomes.

Experiment V.—Rat 47.—A dose of five minims was employed, diluted with an equal volume of normal saline. The index was variable and auto-erythrophagocytosis was observed.

52. In a paragraph headed "The Mechanism of the Destruction of Trypanosomes in the Body" the authors state that they watched the ingestion of the trypanosomes in the living blood. Three trypanosomes in one instance were observed by the side of a phagocyte. In spite of violent contortions, and although other trypanosomes were seen passing freely in the immediate neighbourhood, they appeared unable to get away, and all three were absorbed by the phagocyte within thirty minutes.

53. *Experiment IV* was suggested by the observation that a rat, infected with T. lewisi, and showing staphylococci in the blood, was finally found to be free from trypanosomes, and also recovered from the staphylococcal infection.

Rat 52.—First day, two million dose of staphylococcal vaccine (prepared from the organism from the above-mentioned rat), sixth day, ditto. Succeeding doses three and a half and four million. First dose was followed by a great fall in the index. There was a temporary rise after the second, and again after the third dose. Succeeding doses (three) caused a decrease until on the forty-second day no trypanosomes were seen. On the forty-seventh day, however, they were numerous again.

54. *Experiment VII and VIII* were similarly performed on Rats 61 and 62. The index in both remained at a high level after the injections of the vaccine.

55. The conclusions are reached, that the results were almost entirely negative, and that trypanophagocytosis occurs, by which means large numbers of trypanosomes may be destroyed.

56. Another paper dealing with the subject of Trypanosomiasis, "Auto-erythrophagocytosis as an Aid to the Diagnosis of Trypanosomiasis," is reprinted from the Journal of the London School of Tropical Medicine, Vol. II, Part III, pp. 212 to 215. The authors divide their cases into three groups, A, B, and C.

57. *A.*—Those in which the leucocytes containing the red cells were present in the peripheral blood of infected animals which later showed trypanosomes:—

A monkey injected with trypanosomes, closely resembling T. gambiense, showed erythrophagocytosis on the fifth day, and trypanosomes on the seventh.

A mouse, injected with similar trypanosomes, showed intense ingestion of erythrocytes on the eighth day, and trypanosomes appeared on the ninth day.

A goat, injected as above, showed erythrophagocytosis on the eleventh day, and trypanosomes were first seen on the thirty-third day.

58. *B.*—Those in which auto-erythrophagocytosis and trypanosomes appeared in the blood at the same time at some period of the disease:—

A rat injected with trypanosomes resembling T. duttoni; third day, a few trypanosomes. Fifth day, trypanosomes and ingested red cells; sixth to twelfth day, no parasites but scanty erythrophages.

A rat caught infected with T. lewisi. Ten days later scanty erythrophagocytosis.

A rat caught infected with T. lewisi. Second day, phagocytosis marked.

A rat caught infected with T. lewisi. Third day, ingestion of several red cells observed.

A mouse caught infected with a trypanosome closely resembling T. duttoni. Second day, one red cell seen ingested, a few trypanosomes. Fourth day, more phagocytosis, trypanosomes numerous.

A monkey inoculated with a trypanosome closely resembling T. gambiense. Tenth day, trypanosomes appeared, and on eleventh day erythrophagocytosis also appeared.

59. *C.*—Cases in which erythrophagocytosis was seen after the disappearance of the trypanosomes:—

The first rat in Group B falls under this group also.

60. The number of red cells ingested by a single leucocyte varied from one to twelve or even more.

An important observation is made, that many leucocytes were met with which showed granules apparently derived from ingested red cells, and this may be one possible source of the pigment so frequently observed in the Large Mononuclears in Malaria.

61. The authors put forward the suggestion that erythrophagocytosis may be of material aid in the diagnosis of Trypanosomiasis, in which disease trypanosomes cannot always be demonstrated in the peripheral blood, and more especially in natives, where it is sometimes impracticable to obtain gland-juice or cerebro-spinal fluid.

62. There is also a paper by the same authors on "A Case of Equine Trypanosomiasis, characterised by the occurrence of Posterior Nucleated Forms," in the Journal of Tropical Medicine and Hygiene, Vol. XVI, No. 22, 15th November, 1913, pp. 348 to 349.

63. A series of blood slides was sent to the Medical Research Institute by Dr. A. Hutton from Accra. The films were taken from two horses. The parasites, which were much more numerous in one animal than in the other, were of the T. brucei (pecaudi) type. It was observed that posterior nucleated forms were plentiful. A thousand trypanosomes were examined in the smear from the more heavily infected horse, according to the procedure of Blacklock, and it was found that 4·8% of the parasites showed a posteriorly-situated nucleus.

(c) YELLOW FEVER.

64. Dr. Harald Seidelin, of the Liverpool School of Tropical Medicine, at the time of my arrival was engaged on Yellow Fever investigation, having been specially sent out by the Commission. On his invitation we worked together, mainly on the transmitted disease in guinea-pigs. Our observations, which were unfinished at the end of the year, are still *sub-judice*.

65. A considerable amount of work was done for the Yellow Fever Commission, the results of which will, no doubt, be published in due course, when the members of the committee have considered them. This work consisted in the classification and arrangement of the case-cards sent in by Medical Officers from the various stations where the illnesses occurred, and also the examination of a very large number of pathological specimens and blood films.

66. Dr. Scott Macfie and Dr. Johnston obtained the permission of the Commission to publish the results of their work. Their paper is entitled "Experiments and Observations on Yellow Fever."

It was first reprinted from the proceedings of the Royal Society of Medicine, 1914, Vol. VII (Medical Section) pp. 49 to 67, and was afterwards published in the Bulletin, Yellow Fever Bureau Vol. II, No. 2, April, 1914, pp. 121 to 144.

67. A short account is given of the symptoms in the human cases of Yellow Fever which occurred in Lagos from May till November, 1913. Four cases were in Europeans, three in Syrians, and sixteen in Natives. The classical features of the disease were observed in the former two groups, but the symptoms were variable in the natives, and in practically all of them the illness ran a mild course.

68. Blood films from nineteen cases of Yellow Fever, in the human subject, were examined. Parasites, which the authors conclude are Paraplasma flavigenum (Seidelin), were found in sixteen. The films in the remaining three cases were unsuitable for examination.

69. The parasites were scarce in some cases, but in others they were not so. They were more common in the natives than are malarial parasites during a mild attack of malaria in these subjects. The authors were able to examine the blood daily for twelve days in one case. Parasites were found on eleven days and they persisted after the subsidence of the fever. The opinion is given that the constancy with which the Paraplasma flavigenum is found in the blood of the Yellow Fever patients is a valuable aid to diagnosis. It was impossible to trace out any definite cycle of development, but the several types of endoglobular bodies met with were constant.

70. Fourteen guinea-pigs were inoculated from eleven patients. One or two drops of blood, diluted with 1% Sodium Citrate solution, were injected subcutaneously in each instance. Ten of the animals showed a definite febrile reaction, whilst three other guinea-pigs inoculated in a similar way with normal human blood showed no reaction. Four typical temperature charts are reproduced, the readings having been taken per rectum daily at 5 p.m.

71. Three guinea-pigs, all of which had shown a marked febrile reaction, died suddenly on the 16th, 20th and 28th day after inoculation, respectively. The temperature had returned to normal some time previously in each case. All three showed acute congestion of the liver and kidneys, two had some excess of peritoneal fluid, the urine obtained from the bladder in two contained albumen, and the mucous membrane of the stomach was congested in one. A fourth guinea-pig, killed on the sixth day of illness, showed acute congestion of the liver, kidney, and lungs.

72. Paraplasma flavigenum was found in the peripheral blood of all the animals except one, and this guinea-pig showed auto-erythrophagocytosis.

73. A sub-inoculation was made in one case, resulting in a febrile reaction, with Paraplasma-like bodies present in the blood.

It should be noted that the day of disease, in the human cases from which the blood was taken, varied from the first to the eighth.

74. Five dogs were similarly inoculated with Yellow Fever blood from human cases. No febrile disturbance was observed in any, but Paraplasma-like bodies were found afterwards in the blood of four. One dog died from distemper and mange, but the other four recovered. Eleven stray dogs from Lagos were examined at the same time. Babesia-like or Paraplasma-like bodies were found in the blood of two. One of these dogs was in a dying condition when found. The animal had passed tarry stools, and at the *post-mortem* the liver was found to contain fat, whilst the kidneys showed the signs of acute nephritis.

75. Two white rats were also inoculated from the human cases. Only one reacted with fever, but Paraplasma was found in both.

76. The blood of six fowls, bought in the market, was examined, but no parasites were seen.

77. Atoxyl was tried therapeutically in one case with a control. No marked difference in the course of the illness in the two guinea-pigs was noted. The dose of the drug was one-fiftieth of a grain subcutaneously.

78. A cultivation experiment was made in one human case by the method of Bass, but no growth of parasites was observed.

79. The procedure for the Wassermann reaction was carried out in two cases according to the technique of Fleming. One was done on the sixth and the other on the tenth day of illness, and both were negative.

80. Several tables, some charts, and a very fine coloured plate by Miss Rhodes, showing the various forms of the parasite, complete the paper.

(*d*) POROCEPHALIASIS.

81. Dr. Macfie and Dr. Johnston published "A Note on Five Cases of Porocephaliasis in Man, from Southern Nigeria," in the *Lancet*, 15th November, 1913.

82. The specimens and notes on which the paper is based were received, three from Dr. A. H. Wilson from Itu, the Ahoada district, and Degema, one from Dr. J. D. Finlay at Ibadan, and one from Dr. Currie at Calabar.

83. The age in four of the cases was over forty years and the fifth was aged about seventeen years. The parasites were discovered at the *post-mortem* in all the cases.

84. The first occurred at Ibadan. The porocephalus larvæ were found just under the peritoneal covering of the liver; a few more were seen in the substance of the organ, and some were lying free on the under surface.

85. The second case came from Calabar. A single larva of Porocephalus armillatus was found encysted at the edge of the liver.

86. The third case was met with at Itu. Ten or twelve encysted larvæ were recovered from the liver and a lesser number from the lungs.

87. The fourth case came from the Ahoada district. There were five encysted larvæ in the liver and the peritoneal covering showed a number of cicatricial patches.

88. The fifth case was in a Kroo-boy at Degema. There were larvæ in the liver and one was found in the mesentery.

89. Drs. Macfie and Johnston think that sufficient evidence of disease was detected at the necropsy on the first two cases to account for death, apart from the presence of the larvæ. The other three cases, however, were different. The clinical picture was the same in all three, progressive weakness, œdema and dyspnœa; the pathological findings were the same, and there were no definite signs, apart from the presence of the larvæ, to account for the illness.

(*e*) SPIROCHAETOSIS.

90. A fatal disease amongst fowls was investigated by Drs. Macfie and Johnston and the results were published as "A Note on the Occurrence of Spirochætosis of Fowls in Southern Nigeria," in the Annals of Tropical Medicine and Parasitology, Vol. VIII, No. 1, 21st April, 1914, pp. 41 to 46.

91. The disease was studied in Muscovy ducks and fowls, sent from Iju, near Lagos, by the late Mr. Harris. The signs of the affection are given—fever, diarrhoea, anæmia, paresis, and somnolence. After death the body was found to be wasted, and the tissues pale. The spleen was always considerably enlarged, but the outstanding feature was the enlarged liver, which showed, in addition, necrotic patches.

92. The spirochæte appeared to be very similar to that described by Balfour in fowls in the Soudan. The parasites varied greatly in length, 9 to 27 microns or more, and the spirals varied in number, from 3 or 4 to 12 or 14. The latter, however, appeared to consist of two spirochætes, united by a fine filament. The spirals were either close or open. Both ends of the body tapered, and one end was often seen curled up. In some individuals chromatin dots were distinct. Breaks or gaps in the central core were also observed. Some of the parasites appeared to be made up of two spirochætes, in an attitude suggestive of longitudinal division.

93. Amongst the blood changes observed, large vacuolated mononuclears are noted, and also ingestion of the red cells.

94. A rat was inoculated subcutaneously from a Muscovy duck. Although countless numbers of Spirochætes were present in the blood of the bird, none appeared in the blood of the rat, and no signs of illness developed.

95. Atoxyl, three fiftieths of a grain, and Salvarsan, 0·002 gram, were administered subcutaneously in the treatment of disease in fowls. Two out of three birds treated with the former recovered, as did also the two which were injected with the latter.

96. The "After-Phase" bodies of Balfour are discussed. The authors state that they did not succeed in convincing themselves of their occurrence. The article concludes with a discussion as to the identity of the particular spirochæte found. The belief is expressed that the parasite is morphologically, and in its pathological effects, similar to Sp. marchouxi.

(*f*) LARVICIDES.

97. Some experiments with Stegomyia larvæ are described in "A Note on the Action of Common Salt on the Larvæ of Stegomyia fasciata," in the Bulletin of Entomological Research, Vol. IV, February, 1914, pp. 339 to 344, by Dr. Macfie.

98. The experiments were undertaken in order to ascertain the value of common salt as a larvicide, and it was proposed to investigate the nature of its action as regards (*a*) the effect of the hypertonicity of the solution directly on the larva, and (*b*), the effect of the salt on the natural food supply.

99. Common salt was chosen because of its general applicability, and the larvæ of S. fasciata were used because their breeding places, which are mostly in water-pots in the native compounds, are easy of access, and also because of the importance of S. fasciata in a country where Yellow Fever is endemic.

100. The salinity of the water, in which the larvæ are found in nature, was first obtained by analysis of a number of samples. The average was found to be 0·012%, NaCl and the highest was 0·019%.

101. A number of experiments were then performed with graded percentages of salt, and in the first instance clean water was used in order to eliminate the factor of precipitation. The results are given in Tables. A series of experiments was also carried out with Alum.

102. The results of the work showed that in solutions of 2% and upwards the action of salt on the larvæ is due to the hypertonicity of the solution. When less concentrated solutions are used, the destruction of the natural food supply would appear to have some influence in bringing about the death of the larvæ. Very young larvæ appeared to be more adaptable than the older ones to alterations in the strength of the salt solution. Alum had no peculiar effect.

103. The author considers that it would be of great advantage to use salt in such amount as would give a strength of 2% to the household water-pots in Lagos, as a larvicide in preference to kerosene.

104. A short note is added giving the results of certain experiments, which showed that pupæ of S. fasciata may hatch out in damp soil which is exposed to an intermittent flow of water, such as might be found in the hollows of the ground immediately under the taps of the rainwater tanks.

B.—ROUTINE EXAMINATION OF SPECIMENS.

105. General clinical and other work, including the examination of blood films, of paraffin sections, and the analysis of water, etc., was always undertaken as opportunity afforded.

106. It was occasionally necessary to postpone such examinations for days at a time, when the amount of our experimental work was pressing.

As previously mentioned, many of the specimens received formed most valuable material for experimental research.

107. Thirty-five stations contributed supplies, namely, Accra, Afikpo, Agbor, Ankpa, Aro, Badagry, Bathurst, Bende, Benin City, Bonny, Brass, Calabar, Degema, Deoba, Eket, Forcados, Ibadan, Ibi,

Ikot-Ekpene, Itu, Kano, Lagos, Lokoja, Obudu, Ogoja, Okigwi, Okwoga, Onitsha, Otaminnie, Owerri, Sapele, Wamba-Jemmao, Warri, Wushishi, and Zungeru.

(*a*) CLINICAL PATHOLOGY.

108. This included the examination of smears from blood, from fæces; from ulcers and from discharges generally, and also from organs; the analysis, chemical and microscopical, of urine, the cutting and staining of sections from tissues, and sero-diagnostic work (Widal's and Wassermann's reactions).

109. Seventy-six blood smears from human cases were received. Frequently these were so over-grown with moulds, due to the dampness of the air, and the long journey, that a satisfactory examination was impossible. Filarial embryos, trypanosomes, malarial Plasmodia, and the Paraplasma flavigenum, were the parasites found. A differential leucocyte count was always made in the more interesting cases. A number of the films were taken from cases of Blackwater Fever, and the results of the examinations of these will be found in the Annual Report on that disease for 1913, in a separate publication.

110. Twenty-seven smears from fæces were examined, and the protozoa found included Entamœba histolytica, Lamblia intestinalis, Trichomonas, and Spirochætes.

111. Thirteen organ-smears were received, thirteen smears from ulcers, four vaginal smears, and three from gland juice.

112. Seven samples of blood or of serum were sent in for the Widal reaction. One was positive to Bacillus typhosus, and another agglutinated Bacillus paratyphosus A.

113. Four specimens of urine were received for the examination for ova, casts, etc.

114. Pathological material in bulk for the museum, or in small sections for diagnosis and report, included thirty-five specimens of liver, twenty-eight of spleen, twenty-five of kidney, ten of lung, seven of myocardium, five of brain tissue; four of stomach, four of glands, and one each of testis, ovary, pancreas, small intestine, omentum, appendix, and a portion of tibia.

115. The most interesting conditions observed in these were several examples of Porocephalus infection of the liver (see paras. 81–89), and also another liver which presented a remarkable honeycombed appearance. On microscopical examination, drum-stick bacilli were found in large numbers.

116. Seven malignant tumours were included among the tissues, five sarcomata, one adeno-carcinoma, and one scirrhus cancer.

There were three simple tumours, one fibroma, one fibro-adenoma, and one simple hypertrophy of a lymph gland.

117. Many blood smears, and pathological material generally, have been received, obtained for the most part from the slaughterhouses.

118. Forty-two blood smears from cattle showed the following parasites, Babesia in two, Trypanosoma vivax (cazalboui) in one and T. brucei in another.

119. There were twenty films from sheep and one showed Babesia, and another T. vivax (cazalboui).

120. Blood films from goats numbered nineteen. Babesiac were present in one.

121. A series of blood films from four horses showed T. brucei in two and T. vivax (cazalboui) in a third. A paper was written (see paras. 62 and 63) on one set of these specimens.

122. Trypanosoma pecorum was found in the blood of one pig out of three animals.

123. Five live guinea-pigs, inoculated by the sender with blood from cases of trypanosomiasis, were forwarded to the Institute, so that some interesting series of experiments were carried on.

(*b*) Water Analysis.

124. Twelve samples of water were sent in for bacteriological analysis. A long journey of weeks without ice-packing, rendered the analysis, in some cases, rather misleading.

(*c*) Entomology.

125. Several valuable collections of blood-sucking insects were sent in for identification, or for inclusion in the museum. They contained, amongst other insects, Glossinæ, Tabanidæ, numerous mosquitoes, sand-flies, ticks, and fleas.

(*d*) Helminthology.

126. Various parasitic worms from human sources were received, including Ascaris, Cestodes, Loa loa, Dracunculus medinensis, and Ankylostomes. An interesting series of worms has also been obtained from animals killed at the slaughter-houses.

127. Several photographs of interesting or rare conditions, pathological or otherwise, were added to the museum.

GENERAL.

Special Investigators.

128. Dr. R. T. Leiper, Helmenthologist to the London School of Tropical Medicine, made some use of the resources of the Laboratory during the earlier part of the year.

129. Dr. Harald Seidelin, of the Liverpool School of Tropical Medicine, worked at the Institute for the last three months of the year. He was engaged exclusively on Yellow Fever, having been appointed to investigate that disease by the Yellow Fever Commission

Assistance from Medical Officers.

130. Dr. L. H. Booth and Dr. R. C. Macpherson were both attached to the Laboratory for a period, and in spite of several interruptions for outside work, they rendered valuable assistance.

Additions to the Staff.

131. Amongst additions to the Staff must be noted the appointment of a Laboratory Attendant under the auspices of the Yellow Fever Commission. This post was held by Sergeant F. G. Phipps of the R.A.M.C., from the beginning of June until the end of the year. He soon proved himself to be an indispensable factor in the work

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